Griffith Memorial Prize

STUDIES IN THE DIMENSIONS OF ERYTHROCYTES OF MAN

BY

HEMENDRA NATH CHATTERJEE, M.B.

Griffith Memorial Scholar, Darbhanga Research Scholar, Kalyankumar Mukherjee Research Scholar, Calcutta University; Officer-in-charge, Inquiry on Bone-Marrow and Secondary Anaemias, Indian Research Fund Association.

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PREFACE

The present monogram has been an offshoot of the work on the Anaemia of Pregnancy which was undertaken by the writer under the auspices of the Maharaja of Darbhanga Research Scholarship of the Calcutta University. When taking up the study of the above condition, the writer was confronted with the absence of known reliable data about the haematology of the normal Indian Women. The spade work of finding out the normal data had, therefore, to be first performed.

But in this again the difficulty arose that the methods were liable to give different values for the same identical corpuscular dimensions. There was moreover the handicap that the same procedure would give fairly divergent values under slightly different conditions. Consequently keeping of an open mind was imperative in the comparative study of the different methods in normals as well as in pathological conditions.

When an assessment of the above factors is made it would be found that the study of the red cell corpuscle may give an invaluable data about the pathology, diagnosis and prognosis of different diseases.

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DEPARTMENT OF PATHOLOGY,
CARMICHAEL MEDICAL COLLEGE, CALCUTTA. } H. N. C.

STUDIES IN THE DIMENSIONS OF ERYTHROCYTES OF MAN

Although the value of the determination of the size of red blood cells is now recognised by the Clinician and Haematologist everywhere, the earlier work on the subject seems to have been neglected.

Thus as early as seventeenth century Leeuwenhoek measured the diameter of erythrocytes in man by comparing them with grains of sand and calculated that each corpuscle was 3,000th of an inch or 8·3 microns in diameter. Later observers, however, with the help of more accurate measurements have found that the average size of the red cell is 7·6 microns.

Dr. James Jurin attempted to give the exact figure for the diameter of red blood cells more than 200 years ago ; his methods were naturally crude and in his first attempt he gave the figure 7·75 μ as the diameter of red cell which he later changed to 12·5 μ.

Welcker (1863) made careful measurements of the diameter, thickness and volume of corpuscles. He made plaster models and fashioned them in the shape of red blood cells. According to his calculations the average volume of the red cell was

72 cubic microns, a figure which we now know to be too low.

Mallasez (1889) also worked out the mean diameter of the red blood cells. He seems to be the first to have attached any significance to the variations of red cell diameter.

Amongst the later workers whose findings have contributed greatly towards the application of the size of red cells to clinical medicine, may be mentioned Hedin and Blix (1890-91) in connection with the invention of Haematocrit ; Herz (1893) for the clinical application of the same ; Capps (1903) for finding out and laying stress on the Volume Index instead of the volume alone ; Price Jones (1910) for the use of Camera Lucida for determination of the diameter and for his distribution curve ; Pijper (1924) for the revival of the diffraction methods for finding out the size of small objects including red cells ; Emmons (1927) for the developement of the Eriometer in which the diameter of the cells can be read off directly ; Eve (1929) for devising a similar but cheap instrument working on the same principles ; Wintrobe (1927) for devising a very useful Haematocrit ; Van Allen (1925) for his capillary Haematocrit in which the finger blood can be used for determination of cell volume ; and a host of other workers.

Different methods have been used for the determination of the relative cell mass : Stewart (1919) determined the cell volume from electrical conductivity of the plasma ; Alder (1918) has used Viscosimeter for a similar purpose ; Bliebtreu and Bliebtreu (1892) determined the percentage of cells in a given volume of blood from the nitrogen content of the plasma.

But the various methods that are at present in clinical use for the determination of the volume and the diameter of the cell may be stated to be the following :—

1. *Haematocrit Method*

Haematocrit was first invented by Blix and Hedin (1890-91). For accurate determinations an anti-coagulant is used which at the same time does not change the volume of the cells. The reagents usually used are Heparin, Hirudin or 1·3% solution of sodium oxalate. Many different varieties of Haematocrit have been used such as that of Wintrobe (1925), Bonniger (1909), Van Allen (1925), and several others.

In all these instruments a given volume of blood is centrifuged until packing is complete. The volume of cells present in 100 c. c. of blood is obtained from the Haematocrit reading ; the number of red cells present per c.mm. is also counted. Mean corpuscular volume or the volume of each individual corpuscle is then easily calculated. Thus if X denotes the volume of packed red cells in 100 c.c. of the tested blood, Y the number of red blood cells per cubic millimetre, then the mean corpuscular volume would be

$$\frac{X \times 10^{12}}{Y \times 10^{11}} \text{ cubic microns.}$$

The figures obtained by us in 135 normal persons living in Bengal are given below :

Highest	100	cubic	microns.
Lowest	71	,,	,,
Average	90	,,	,,

2. *Camera Lucida Method* (*Price Jones*)

In this method the usual procedure consists in sketching out the outline of 500 red cells by the help of the Camera Lucida arrangement, measuring them and finding out their mean. The tube length of the microscope, the eye-piece and the inclination of the mirror are carefully adjusted by means of a special slide marked with μ divisions and a good millimeter scale so that one μ division under oil immersion would exactly correspond with one millimeter drawn on paper. Two diameters of each cell are measured at right angles, and the mean noted. After 500 cells are thus measured the mean can be found out and a frequency curve plotted, the abscissa denoting the diameter in microns and the ordinate the percentage number of cells.

As the majority of the cells in a normal person are about 7·6 μ in diameter the curve obtained in such a case would be a steep one with a narrow base and apex high up in region of 7 μ. On the other hand, in anaemic conditions there is less uniformity in the size of cells which may vary from small sized ones to those with diameters of 10, 11 or 12 μ. Hence the base will be wider and the apex flattened. The curve will be deviated either to the right or to the left, accordingly as the majority of the cells are greater or less than normal size. A macrocytic or microcytic type of curve would be obtained as shown in the accompanying figure (Fig. 1).

The figures obtained by us as the mean corpuscular diameter from 135 normal persons by the Camera Lucida method are given below :

Highest ...	... 7·9 μ
Lowest ...	... 6·6 μ
Average ...	... 7·4 μ

3. *Diffraction Method*

Dried blood films are used for this purpose, the corpuscular film acting as a diffraction grating. Emmons' Eriometer or Eve's Halometer utilises the above principles of diffraction as has been mentioned previously. The average diameter is calculated by referring to a specially worked out table.

The average obtained by us in 135 normal persons with unstained coverslip-drawn films is $7{\cdot}3\mu$.

Besides the above methods, direct mesurement of the cells by means of the specially graduated slide and the eye-piece may be feasible; but it would entail such an amount of labour and time as to preclude its use in practical clinical work. The diameter can also be calculated from the chart devised by Warburg (Fig. 2). The two other data necessary for this are the corpuscular volume and the number of cells per c. mm. This method takes it for granted that the thickness of the corpuscles is constant, a fact which we know is not true. Consequently, the figures are not expected to be accurate in all cases.

Advantages and Disadvantages of the different Methods

The method of Price Jones has the unique advantage of giving not only the average diameter but what is equally important, it presents the nature of the actual distribution of the different sizes of the cells; in other words, it gives a differential count of the red blood cells classified according to size. The curve of Price Jones at a glance gives a complete and vivid idea about the whole state of affairs about the diameter of cells, so far as qualitative information is required.

The Halometer, on the other hand, gives the results in a much shorter time. The reading is easy and the results can be calculated in a few seconds. But at the best it can give some idea only, about the average diameter of the cells. It is unable to present any information about the relative distribution of the different sized cells.

But very serious objections to both the above-mentioned methods are the following :—

1. Both the methods take into account the dimensions of the corpuscles in one plane only. The thickness or the 3rd dimension is absolutely ignored.

2. It has been recently pointed out by Napier (1936) that in the Camera Lucida method the same cell might show significant differences if moved in the different parts of the microscopic field. The mean size of the cells increase from the centre to the periphery of the microscopic field, thus bringing into play a very significant source of mistake due to refraction. It is possible that the above source of error is similarly present in the Halometer—the cells of the periphery behaving as diffraction gratings in a different way from those at the centre.

3. There is a distortion of the shape of many of the cells during the drawing of the film even when the same is done by means of a coverslip. Many of the cells lose their circular outline in the dried smear specially in cases of anaemia and the mistake can be corrected in the Price Jones method,

though partially, by measuring two diameters at right angles and taking their mean.

4. In addition to all the above sources of error the pressure exerted during the drawing of the film and the time taken in doing this may materially change the size of the cell in the dry films; here, by means of sheer pressure we are converting a biconcave elastic colloidal bag into a hardened and flattened disk; if the films are stained after artificial fixation the changes produced will be all the more gross. The above statement will be apparent from the following tables (Tables I and II), obtained from the corpuscular examination of two healthy and two anaemic persons. The different preparations, *e.g.*, the coverslip-drawn preperations (long film and short film), slide-drawn preparations (long film and short film), the moist films as well as the Haematocrit (Van Allen) were made from the identical fingure puncture in every case.

TABLE I

Values of Mean Corpuscular Diameter of Healthy cases in microns

		Moist film.	Unstained dry film.				Stained dry film.				Mean corpuscular volume.	Mean corpuscular Diameter (Warburg's Chart).
			Cover-slip-drawn short.	Cover-slip-drawn long.	Slide-drawn short.	Slide-drawn long.	Cover-slip-drawn short.	Cover-slip-drawn long.	Slide-drawn short.	Slide-drawn long.		
Case I	Camera Lucida	7·2	6·7	6·7	7·4	7·1	6·3	6·5	7·2	6·5	75 c. microns	7·2
	Halo-meter	7·4	7·4	7·3	7·4	7·3	7·3	7·0	7·0	7·1		
Case II	Camera Lucida	7.6	7·1	7·3	7·4	7·1	7·1	7·1	6·8	7·2	92·7 c. microns	7·5
	Halo-meter	7·7	7·4	7·3	7·6	7·3	7·1	7·1	6·8	7·2		

TABLE II

Values of Mean Corpuscular Diameter of Anaemia cases in microns

		Moist film within 1 hour.	Unstained dry film.				Stained dry film.				Mean corpuscular volume.	Mean corpuscular Diameter (Warburg's Chart).
			Cover-slip-drawn short.	Cover-slip-drawn long.	Slide-drawn short.	Slide-drawn long.	Cover-slip drawn short.	Cover-slip-drawn long.	Slide-drawn short.	Slide-drawn long.		
Case P.D. age—26 R.B.C:— 3040000	Camera Lucida	7·5	7·2	7·5	7·6	7·8	7·4	7·2	7·4	7·2	80 c. microns	7·4
Corpuscular volume 24%	Halo-meter	7·9	7·8	7·6	8·0	7·6	7·6	7·4	7·7	7·8		
Case—P.B. R.B.C:— 4010000	Camera Lucida	cells distorted	6·8	7·0	7·2	7·1	6·8	7·0	7·1	6·9	70 c. microns	7·1
Corpuscular volume 28%	Halo-meter	cells distorted	7·3	7·6	7·2	7·6	7·3	7·3	7·1	7·4		

A similar difference in the means is obtained by basing the observations on the measurements of a large number of corpuscles (20,000) from two different groups of persons, *viz.*, healthy normals and patients suffering from anaemia as will be seen in Table III.

TABLE III

Average Mean Corpuscular Values in Normal cases in microns

Price Jones Camera Lucida stained slides coverslip-drawn 20,000 cells drawn from healthy women.	Mean Halometer stained slides coverslip-drawn.	Mean Halometer reading unstained slides coverslip-drawn.	Mean corpuscular volume.
6·9	7·1	7·3	90·6 c. microns
	Anaemia cases		
Price Jones curve Camera Lucida stained slides coverslip-drawn average of 20,000 cells of anaemia cases.	Mean Halometer reading stained slides coverslip-drawn.	Mean Halometer reading unstained slides coverslip-drawn.	Mean corpuscular volume.
7·1	7·4	7·7	102 c. microns

From the above tables a rather noticeable, though not absolutely constant, fact presents itself, *viz.*, that there is a distinct tendency for the stained slides to give lower figures those that given by the same slides when unstained. This is perhaps due to the contraction of the red cells from fixation and staining.

One should also bear in mind the other likely sources of error, *i.e.*, due to parallax and to personal factors during the sketching of the cells by the Camera Lucida. It will thus be evident that the different slides give greatly varying figures. Hence accuracy of readings can hardly be expected in dealing with the dried films of biological bodies such as the red blood cells, suspended in a biological fluid where a great many factors are coming into play. Amongst these may be mentioned factors such as the surface tension, the presence of the electrolytes, the elasticity and the resistance of the cell wall, the amount of pressure and the time taken in drawing the films, over and above the mistakes due to parallax and personal variations in making the sketches as well as taking the readings.

From a theoretical consideration, enumeration of the moist cells seems to be the best for taking the Price Jones readings. The cells do not show the great distortions in shape and size as we find in the dry films provided there is no crenation and hemolysis.

A count of 100 cells at half-hour intervals repeated 3 times up to 1½ hours after the initial puncture had given identical results in 6 of our normal cases.

This procedure, however, will be of only limited practical value as the cells get distorted and crenated after some time ; this is specially so in cases of anaemia and the readings from such cases are practically useless.

Therefore when the use of moist film is not practicable it is difficult to decide which slide is to be taken for finding out the mean corpuscular size; in these cases the volumetric method (mean corpuscular volume) is of great use. The advantages of the volumetric method are :

1. It takes into consideration all the dimensions of the red cell. Not only the actual volume of the average cell, but also the haemoglobin contained in each corpuscle can be easily calculated.
2. It is a more uniform method of calculating the average size of the red blood cell.
3. The errors of refraction do not come into play in the volumetric method.
4. It gives a more delicate indication as to the corpuscular changes produced by anaemia than that obtained by diameter alone. This is exemplified by the findings obtained from the following up of a case of Pregnancy anaemia, as shown in Table IV.

TABLE IV

Showing Dimensions of red cells in a case of Pregnancy Anaemia

Case No. XLI

Date.	Mean corpuscular volume in cubic microns.	Diameter in microns obtained from Camera Lucida drawings.	Diameter in microns obtained by Halometer.	
			Unstained slides.	Stained slides.
3.1.36.	116·6	7·8	7·6	7·6
14.1.36.	87·5	7·7	7·8	7·6
22.1.36.	100	7·7	7·3	7·3
31.1.36.	95	7·5	7·4	7·3
1.3.36.	98	6·9	7·3	7·4

According to Haden (1931) a 50% increase in cell volume is obtained when there is an increase of cell diameter by 16%. Hence the cell volume increase is three times more pronounced than the increase of cell diameter. Conversely, with a 50% decrease in cell volume there is a 21% decrease in cell diameter. Hence in microcytic anaemia the cell volume decrease is more than twice as pronounced as the decrease in cell diameter.

For quantitative accuracy the volumetric method, therefore, is the more delicate and accurate one as it seems to be free from the various fallacies. But this should be further reinforced by the very valuable information that can be obtained by the Camera Lucida method.

The moist film is the ideal one for taking the Camera Lucida drawings, but this is not possible in all cases owing to the increased fragility of cells. In these cases the various fallacies of dry films must be borne in mind.

Clinical application of the red cell dimensions (cell volume and cell diameter)

The study of the cell dimensions may be used in clinical medicine, for

(*a*) Diagnosis and Prognosis,

and (*b*) Therapy.

(*a*) Diagnosis and Prognosis.

Unfortunately a final clear cut differentiation and classification of anaemias has not yet been arrived at. The usual methods of classification is to compare the size and the haemoglobin content of the average cell in a particular case with that of the normal.

The various means and indices that are used in present day Haematology about the size and haemoglobin content are given below together with the average figures obtained by us from 135 normal persons residing in Bengal. These are :

(*a*) Mean corpuscular volume in cubic microns :

Highest	Lowest	Average
100	71	90

(*b*) Mean corpuscular haemoglobin or the haemoglobin content of average red cell in micrograms :

Highest	Lowest	Average
46	29	36

(*c*) Mean corpuscular haemoglobin concentration or the mean haemoglobin concentration per cent. per corpuscle :

Highest	Lowest	Average
50%	34%	40%

The Indices are :—

(*a*) Colour Index

or the amount of haemoglobin per cell relative to normal :

average ... 1·1·1

(*b*) Volume Index

or the relation of the average cell to normal

average ... 0·9-1·1

(c) Saturation Index

or the amount of haemoglobin per unit
volume cell relative to normal
average ... 1-1·3

By means of the above means and indices the anaemias are divided into the (1) macrocytic, (2) microcytic and (3) normocytic groups ; and also the (1) hyperchromic, (2) hypochromic and (3) normochromic groups.

Patients with Addisonian anaemia show a macrocytic blood picture. It may be stated in the words of Haden (1931) : "In the absence of increased cell volume the diagnosis of pernicious anaemia is open to question provided the blood picture has not been altered by specific treatment." These cases are usually hyperchromic.

Anaemias of haemorrhagic origin usually show a low volume index (see Table VI). In 10 cases belonging to this group the average volume index was 0·8, although in one extreme case of this group the blood picture was macrocytic and the volume index was 1·2. The study of the cell size may throw a good deal of light on the blood conditions of the other varieties of anaemias, specially if the observations are repeated and the cases are followed for some time. Thus in anaemias due to sprue, Thaysen (1932) found that the size and the colour index varied according to the severity of the case so that the cases were hyperchromic and macrocytic during the bad periods and frequently simple or hypochromic and normocytic during the remissions.

A similar thing happens in cases of a very common variety of anaemia in this country, *viz.*, that associated with pregnancy. One and the same patient, as has been found in the majority of cases examined by us, might pass through the macrocytic or the microcytic stage, the blood picture depending on the amount of anaemia. As the number of cells goes on improving, the size of the cells diminishes from the macrocytic stage and *vice versa*. At first, the case starts as normocytic anaemia; with progressive deterioration the patient passes through microcytic to macrocytic stage, which always means a serious state of the blood condition. This will be apparent from Table V which shows one of our typical cases (Case XIX).

TABLE V

Date.	Number of r. b. c.'s per c. mm. in millions.	Mean corpuscular volume in cubic microns	Volume Index.	Colour Index.
26.11.35.	1·68	53	0·6	0·7
9. 1.36.	1·02	120	1·3	1·5
14. 1.36.	1·01	130	1·4	1·6

With the improvement in the clinical picture the number of cells goes on improving, and along with it the cell volume also shows the changes mentioned above. The same will be evident from the next Table VI in which another of our typical cases is depicted.

TABLE VI

Date.	Number of r. b. c.'s per c. mm. in millions.	Mean corpuscular volume in cubic microns.	Volume Index.	Colour Index.
18.9.	1·09	128·4	1·4	1·7
28.9.	1·96	81·6	0·9	0·9
22.10.	2·37	88·6	0·9	1·0
25.10.	3·20	89	1·0	1·0

The writer has tried to study the same question with regard to the other common varieties of secondary anaemias in this country, *viz.*, those due to Kala-azar, Malaria, Chronic Haemorrhage, Chronic Sepsis and Helminthiasis.

Except the malaria cases, all the three types of anaemias, *viz.*, normocytic, microcytic and macrocytic, have been present in every group. This will be shown in Table VII.

TABLE VII

Causes of anaemia.	Number of cases studied.	Normocytic.	Microcytic.	Macrocytic
Chronic Haemorrhage	10	8	1	1
Chronic Sepsis	10	7	1	2
Malaria	10	10	0	0
Kala-azar	10	7	3	2
Helminthiasis	3	2	0	1

The cell counts of all cases in the macrocytic group was 1·5 millions or less. The writer rather feels that the cases examined are too few ; that the haematology of the secondary anaemias requires a good deal of further study, and that the same investigation would throw a great amount of light not only on the blood pathology of these conditions but would furnish much valuable information about the therapy as well.

The recent literature on the subject is increasingly clarifying the position of the blood pathology of the different anaemias. It may be stated that in addition to those mentioned already, microcytic

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blood picture has been more or less constantly observed with the following conditions :—

1. Idopathic Microcytic Anaemia due to Iron Deficiencies (Minot and Heath, 1932; Price Jones, 1932).
2. Hypochromic Anaemias of infants and children (Mackay, 1931 ; Straus, 1933).
3. Chlorosis (Musser and Wintrobe, 1930).
4. Various secondary anaemias. Owing to a constant increase in the list of this group of anaemias, one would hesitate to enumerate them here. Some of these have already been mentioned. Just like the Anaemia of Pregnancy, it is quite possible that there might be a transition from one type of blood picture to the other and the more severe grades of these would represent a macrocytic picture.

Thus a macrocytic blood picture has been observed by various workers, associated with the following amongst the many conditions :

1. Di-brothrio-cephalus infection (Birkeland, 1933).
2. Gastro-Jejunal fistula (Fairley, Fairley and Kilner, 1931).
3. Gastric Carcinoma (Simpson, 1931).

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4. Anaemia following Gastrectomy (Hurst, 1932).
5. Associated with liver disease (Wintrobe and Shumacher, 1933).
6. Associated with cirrhosis of the liver (Fellinger and Klima, 1933).
7. Sprue (Thaysen, 1932).
8. Coeliac disease (Vaughan and Hunter, 1932).
9. After application of organic synthetic hair dyes (Baldridge, 1935).
10. Lead poisoning (Davidson, 1932).
11. After internal radiation due to radio-active substances (Martland, 1931).
12. Megalocytic and hyperchromic anaemia of infants (Cooley and Lee, 1932).

(*b*) Therapy.

Role of the cell-size determination in therapy (Pernicious Anaemia)

In recent years the treatment of Addisonian anaemia has attracted a great amount of attention from the Hematologists and Clinicians in different parts of the world. This same condition, however, is not so common in this country.

According to different workers the volume has been found to be of the greatest value as a guide to the treatment of pernicious anaemia. This condition is supposed to be due to the want of some specificprinciple ; extracts from liver or the stomache mucous membrance is supposed to supply this important missing factor. If the substances mentioned above are administered in sufficient amounts, the red cell counts, the cell volume, and the volume index return to normal. On the other hand, as Haden (1931) remarks : " If the missing principle is supplied in insufficient amounts, the cell volume remains above normal although the haemoglobin may approach normal. The sign of a relapse is a beginning and progressive macrocytosis. Therefore the determination of the cell volume is very necessary for the proper treatment of pernicious anaemia."

Treatment of Anaemia of Pregnancy

This is still under investigation by various workers, but a great deal of light seems to have been thrown on the subject.

As has already been remarked, the cell volume shows remarkable changes in the different stages of the condition depending on their severity.

In the early stages the condition starts as normocytic which might either be normo- or hypochromic. The next stage is microcytic and might be similarly normo- or hypochromic. It is in these above stages that the role of the iron therapy comes in when it can definitely prevent the downward drift of the condition. This will be evident from the following Table VIII.

TABLE VIII

Showing effect of iron therapy in mild cases of Anaemia Pregnancy

Case.	Date.	Number of r.b.c.'s per c. mm. in millions	Mean corpuscular volume in cubic microns	Volume Index.	Colour Index.
III	6.9.35.	3·4	85·2	·9	1·15
	18.9.35.	4·02	85·0	·9	1·1
XXXI	13.11.35	2·9	76	·9	1·15
	25.11.35	3·4	82·4	·9	1·1

On the other hand, if the condition remains untreated then a macrocytic anaemia sets in. This stage again can be definitely subdivided into two stages :—

(*a*) When the red blood cell count is near about 2 millions.

(*b*) When the red blood cell count is near about 1 million.

In the stage (*a*), liver therapy plays an important part and can greatly improve the condition. This will be evident from our Case XIV (see Table IX).

TABLE IX

Showing improvement with Liver Therapy

Case.	Date	Number of r.b c 's per c mm in millions.	Mean corpuscular volume in cubic microns.	Volume Index.	Colour Index.
...	11.1.36	1·6	111	1·3	1·3
XIV	18.1.36	1·4	110	1·1	1·6
...	1.2.36.	2·2	76	·9	·9

TABLE X

Case. IIA	Date	Number of red cells per c. mm. in millions.	Diameter by Halometer short unstained films.	Remarks.
...	12.8.36.	1·03	8	Liver extract intravenous followed by daily intermascular injections
...	22 8.	·96	8·1	
...	26·8.	·71	8·6	Transfusion 10 ozs. of blood.
...	31·8.	·93	8·4	
...	26.9.	1·37	7·8	Followed by liver extract therapy.
...	17.10.	·64	8·2	
..	18.11.	0·74	7	Transfusion 10 ozs.
...	30.11.	·95	7·6	
...	8.12.	1·3	7·8	

On the other hand, the stage (*b*) indicates a greater disfunction of the bone marrow approaching impending failure. The liver therapy is not of much use in this stage and might be associated with gradually diminishing red cell count. The only remedy that can avert a fatal termination and can possibly tide over the crisis of confinement is blood transfusion which greatly improves the blood condition when the case can be further followed up by liver extract and later on iron. This is shown in Table X.

Treatment of Secondary Anaemias

The treatment of other secondary anaemias will necessarily imply the attention to the specific cause and also the amount and the stage of anaemia, the indications being similar to that mentioned above.

The 3rd Dimension of the Red Blood Cell

It has already been pointed out that both the Halometer and the Camera Lucida present a very great disadvantage in that both give the dimension of the cells in one plane only. The very important third dimension, *viz.*, the thickness, has got to be ignored. This important subject has not received the consideration it requires. Von Boros (1932) and Haden (1935) have laid great emphasis on the same.

Unfortunately there is no direct method of the measurement of cell thickness. According to Ponder (1934), "The only satisfactory method of measuring the thickness is to photograph the cells on the edge while floating in serum and plasma." In five individuals he found the maximum thickness to be $2{\cdot}4\ \mu$ and minimum thickness to be $1{\cdot}02\mu$.

Price Jones et al (1935) have calculated the mean corpuscular thickness by "dividing the mean corpuscular volume by $\pi\left(\frac{D}{2}\right)^2$ $(\pi = 3{\cdot}14)$ which assumes that the cell is a short cylinder, the biconcavities being ignored, an assumption which we know is not correct"

Von Boros (1932) has elaborated a very convenient three dimensions chart based on similar principles from which the thickness can be easily calculated, if the other two factors are known, *viz.*, the mean corpuscular volume and the mean corpuscular diameter.

For clinical purposes the best and fairly accurate method of finding out the thickness would be to find out the mean corpuscular diameter by the Camera Lucida from the moist films and the mean corpuscular volume from the Haematocrit readings; the data can then be referred to Von Boros chart for finding out the thickness. Where, however, the moist film drawings are not practicable owing to the increased fragility of the red cells as in anaemic conditions, Halometric readings of unstained coverslip-drawn short films may be used for the determination of the cell diameter.

The figures of cell thickness obtained by us by the latter procedure in 135 persons resident in Bengal are given below :—

Highest ...	... 2·1 μ
Lowest ...	... 1·7 μ
Average ..	... 1·8 μ

As already mentioned the attention of the Hematologists has been drawn to this important cell dimension very recently and this subject presents a wide field of study. One of the important conditions which is definitely known to be associated with increased cell thickness is Acholuric Jaundice. Thus a blood slide from such a case is liable to be erroneously interpreted as showing a microcytic picture if the diameter alone is measured; the volume of each corpuscle in this condition is greater than normal or normal owing to the increase in the thickness of the cell (Fig. 4).

Investigations on the Hamatology of pregnancy anaemia have revealed a very interesting feature. The increased cell volume in the macrocytic type of cases has been closely associated with cell thickness greater than normal. On the other hand, the increase in the cell diameter has been less constant in these cases and has not been present in all cases of increased cell volume.

Similarly, the microcytic cell volume has been constantly associated with cell thickness which is less than normal. The diameter of the cells has not been microcytic in all these cases.

The above parallelism between the cell volume and the cell thickness in cases of anaemia of pregnancy is observed not only in isolated individual blood examinations but also with repeated observations on the same patient. The above statement will be evident from Table XI.

TABLE XI

Showing relation of cell volume and cell thickness in cases of Anaemia of Pregnancy

Case.	Date.	Mean corpuscular volume in c, microns.	Mean thickness of corpuscle in microns.	Mean diameter from Halometer unstained slides in microns.	Mean diameter from Camera Lucida method stained slide in microns.	Red cell count in millons.
IX	18 9.35.	128·4	2·8	7·6	6·8	1·09
	28. 9.35.	81·6	1·9	7·4	6·4	1·96
	22.10.35	88·6	1·9	7·6	6·7	2·37
XI	20. 9.35	210	3·8	8·0	7·6	0·76
XIII	21. 9.35.	130·4	2·3	8·6	8·1	2·1
XX	1 11 35.	94	2·1	7·6	7·2	1·6
	11.11.35.	111	2·5	7·6	6·9	1·08

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TABLE XI (*continued*)

Microcytic Blood Pictures.

Case.	Date.	Meanmrpuscular volume in c. m^{1}ons.	Mean thickness of corpuscle in microns.	Mean diameter from Halometer unstained slides in microns.	Mean diameter from C mera Lucida method stained slide in microns.	Red cell count in millons.
XXV	7.11.35.	65	1·5	7·4	7 0	2·7
XXIX	26.11 35.	53	1·1	7·4	7·5	1·6
XXV	19.11.35.	64·3	1·5	7·4	6·6	2·7
XIII	10 2.36.	65·2	1·4	7·8	6·8	2·29

Summary

A. The historical evolution of the different methods of finding out the mean corpuscular volume and diameter is discussed.

B. The relative advantages and drawbacks of the different methods are given as well as the average figures obtained in 135 normal persons in Bengal.

C. The clinical application of the above methods are mentioned and their help in the diagnosis, prognosis and therapy in the different types of anaemias are detailed both from literature as well as from personal observations.

D. The methods for finding out the very important third dimension or the thickness of the red cell are given together with average figures obtained in 135 normal persons in Bengal.

E. The changes in cell thickness in Anaemia of Pregnancy are given in detail.

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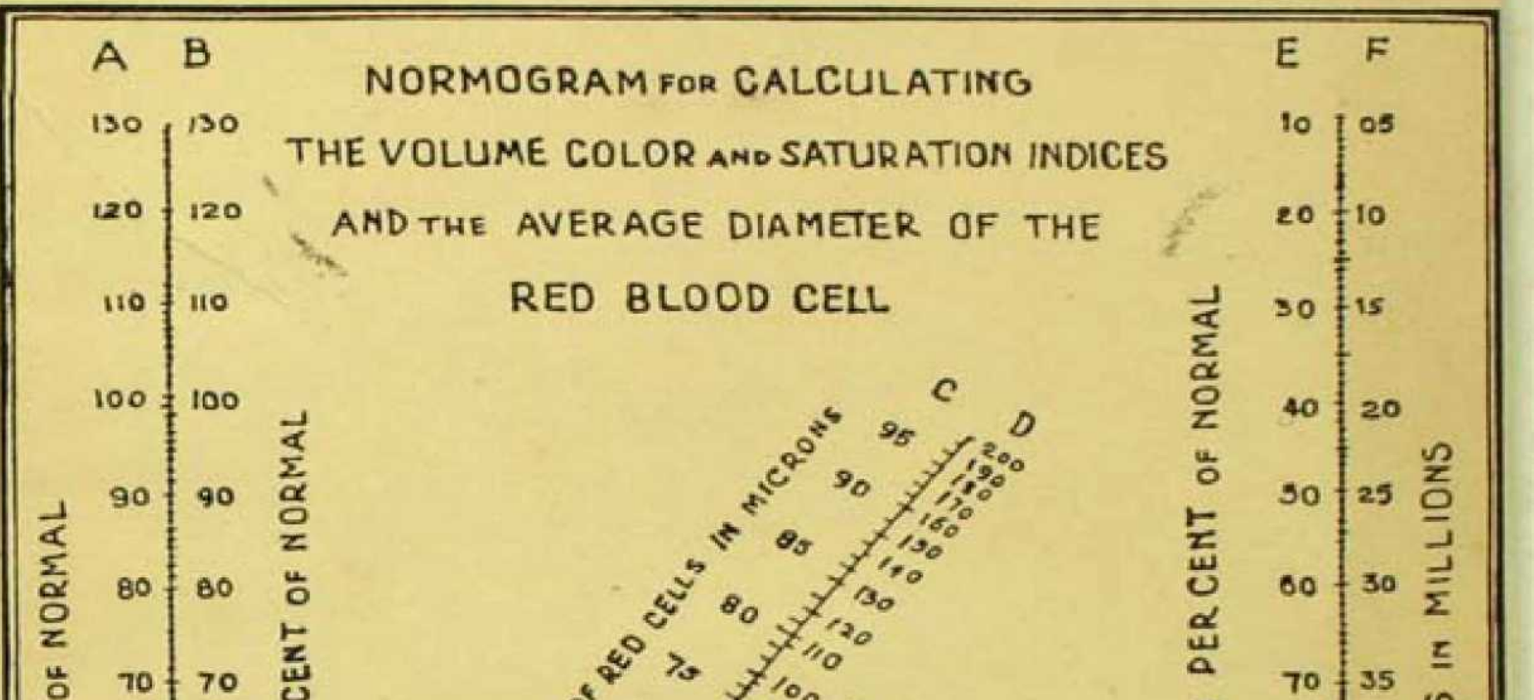

Figure 2. Warburg's Chart

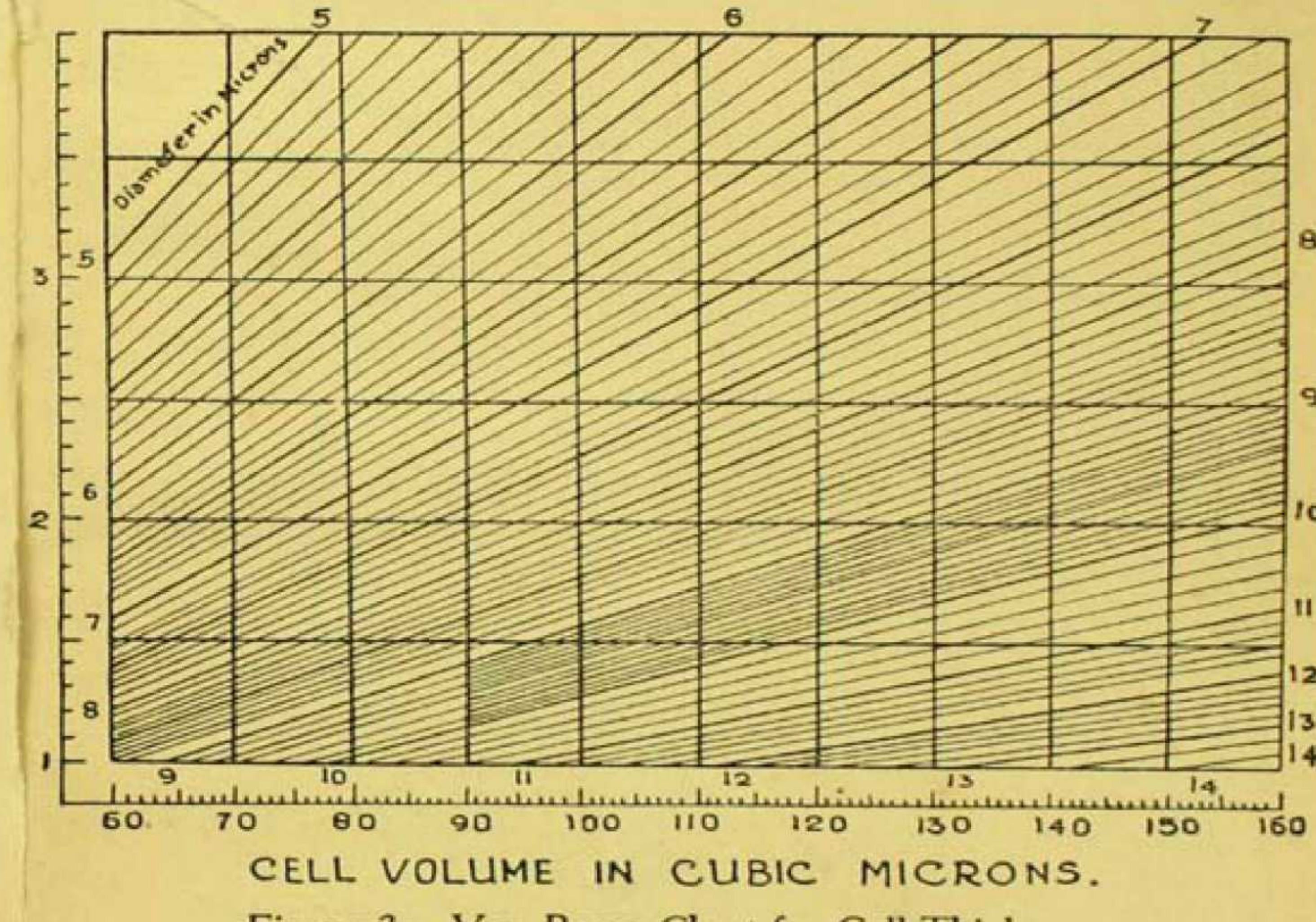

Figure 3. Von Boros Chart for Cell Thickness